Final Report to the Department of Energy

The Respiratory Chain of Alkaliphilic Bacteria

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Goals of the Project Period:

Bacteria that grow non-fermentatively at extremely high pH values confront specific bioenergetic challenges that have evidently led to adaptations in the machinery, i.e. the membrane-embedded energy transducing complexes, of oxidative phosphorylation as well as the lipid composition of the coupling membrane in which those complexes function. The goals of this project were focused on the respiratory chain of extremely alkaliphilic *Bacillus* strains, especially *Bacillus pseudofirmus* OF4. We sought to biochemically characterize the major respiratory chain complexes and, in particular, to examine the terminal oxidases of the respiratory chain, especially the caa_3-type cytochrome oxidase encoded by the *cta* operon.

Summary of Project Accomplishments:

During the final project period, the major accomplishments were:

(i) Gene loci encoding the alkaliphile *bd* –type cytochrome oxidases and *sdh* (Complex II, which had been characterized biochemically during an earlier period of the project) were identified using primers constructed based on emerging genomic sequences from other *Bacillus* species.

(ii) A mutant of *B. pseudofirmus* OF4 with a deletion in the *cta* operon that encodes the caa_3-type cytochrome oxidase was found unable to grow non-fermentatively on malate even though it had highly elevated levels of an alternate (albeit non-proton-pumping) terminal oxidase. That alternate oxidase, one of the two *bd*-type oxidases of the organism, was characterized biochemically.

(iii) Two dimensional gel electrophoresis analyses of cytoplasmic and membrane fractions from cells of *B. pseudofirmus* OF4 that had been grown at pH 7.5 or 10.5 provided evidence for global changes in the proteome as a function of the pH of growth and identified membrane-associated gene products of interest for further study. These included an S-layer that was characterized by electron microscopic as well as genetic studies, and a yet-to-be pursued indication that cycling of lipids may be involved in adaptation to growth at high pH.

Overall Accomplishments of the Project:

The respiratory chain as a whole, the redox poises of its components and several individual complexes of the respiratory chain of alkaliphilic *Bacillus pseudofirmus* OF4 have been characterized as part of this project and, importantly, this project has helped support the development of genetic tools that make *B. pseudofirmus* OF4 the most genetically tractable and, hence, most bioenergetically characterized extreme alkaliphile. Evidence has been obtained for a pivotal role of the cca_3-type terminal oxidase in oxidative phosphorylation, especially at high pH and motifs that may be relevant to that special role have been identified.

Publications not previously reported:

Gilmour, R., Krulwich, T.A. 1997. Construction and characterization of a mutant of alkaliphilic *Bacillus firmus* OF4 with a disrupted *cta* operon and purification of a novel cytochrome *bd*. J. Bacteriol. **179:**863-870. PMID; 9006044

Gilmour, R., Messner, P., Guffanti, A.A., Kent, R. Scheberl, A., Kendrick, N, Krulwich, T.A. 2000. Two-dimensional gel electrophoresis analyses of pH-dependent protein expression of a facultatively alkaliphilic *Bacillus pseudofirmus* OF4 lead to characterization of an S-layer protein with a role in alkaliphily. J. Bacteriol. **182:**5969-5981. PMID: 11029415

No patents arose from this project.